Reference: *Biol. Bull.* **176:** 257–271. (June, 1989)

Ultrastructure and Development of Dimorphic Sperm in the Abyssal Echinoid *Phrissocystis multispina* (Echinodermata: Echinoidea): Implications for Deep Sea Reproductive Biology

KEVIN J. ECKELBARGER, CRAIG M. YOUNG, AND J. LANE CAMERON

Division of Marine Sciences, Harbor Branch Oceanographic Institution, 5600 Old Dixie Highway, Fort Pierce, Florida 34946

Abstract. Mature males of the abyssal echinoid *Phrissocystis multispina* produce two types of sperm including a euspermatozoon typical of echinoids, and a paraspermatozoon, which is bipolar-tailed. The structure of the testis and most features of spermatogenesis are similar to that of other echinoids. Development of both sperm types is identical until the spermatid stage when the nucleus of the paraspermatozoon undergoes chromatin reduction. Both sperm types have acrosomes typical of other echinoid sperm. However, we never observed a Golgi complex during any stage of sperm differentiation so the origin of the acrosome is unclear. Both the distal and proximal centrioles are involved in the formation of an anteriorly and posteriorly directed flagellum in the paraspermatozoon. Mixtures of both sperm types tend to clump due to the entanglement of sperm axonemes in the paraspermatozoon flagellum. Although the function of the paraspermatozoa is unknown, they may play a role in facilitating fertilization through the reduction of euspermatozoon diffusion during spawning. This study reports only one of several recently discovered reproductive adaptations associated with deep-sea habitats.

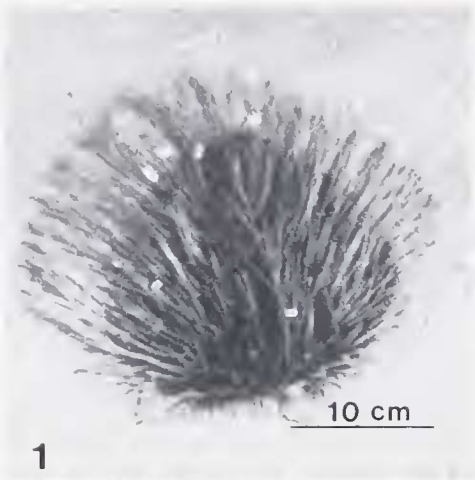
Introduction

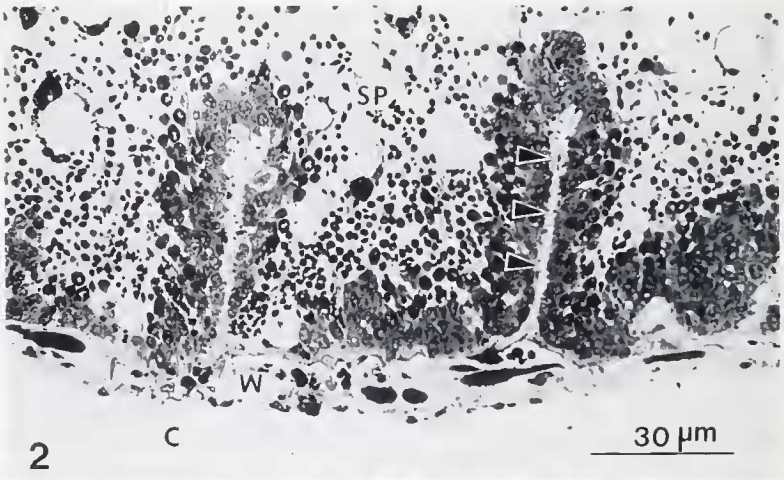
Echinoderms are often the most abundant and diverse macrofaunal organisms in the abyssal and bathyal zones of the ocean (Billet and Hansen, 1982; Pawson, 1982) and the reproductive cycles of many deep-sea species have been investigated (see Lightfoot *et al.*, 1979; Tyler *et al.*, 1982; Tyler, 1988). Until now, however, technology has restricted the study of deep-sea reproduction largely to the analysis of paraffin sections of preserved gonads collected from trawls or dredge collections (George and Menzies 1967, 1968; Rokop, 1974, 1977; Ahfeld, 1977; Pain *et al.*, 1982a, b; Tyler and Gage, 1982, 1983, 1984; Tyler *et al.*, 1982). Little is known about gamete structure, fertilization biology, or development of any deep-sea species due, in part, to the difficulty of obtaining live specimens in good condition.

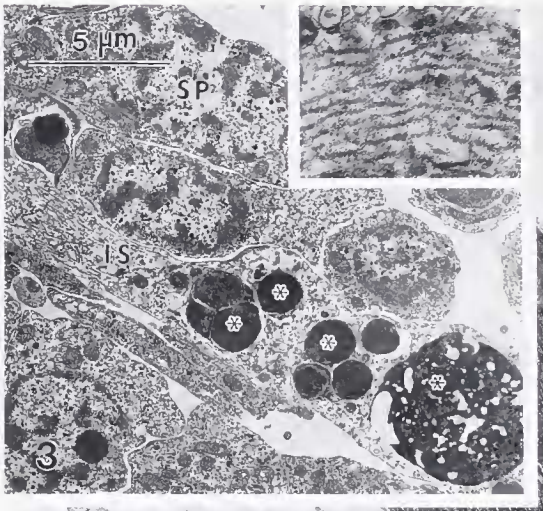
Echinoderm sperm have been extensively studied and, in comparison to most invertebrate sperm, are morphologically conservative (Chia and Bickell, 1983). This study describes the ultrastructural features of spermatogenesis in *Phrissocystic multispina,* a deep-sea spatangoid collected in Hawaiian waters with the Pisces V submersible. Males of *P. multispina* have dimorphic sperm, the first recorded occurrence in the Echinodermata, and although one type of sperm is typical of echinoids, the other is bipolar-tailed, a feature which is structurally rare among metazoan sperm.

Animal sperm morphology is strongly influenced by the mode of fertilization and the environment into which the sperm are released during spawning (Franzen, 1956, 1970). The appearance of dimorphic, aberrant sperm in *P. multispina* and unusual sperm in several other deep-sea echinoderms (Healy *et al.*, 1988; Eckelbarger *et al.*, in press) suggests that unique selective pressures may be present in the abyssal zone of the deep sea which are absent from shallow water habitats. The present paper describes the ultrastructural features of spermatogenesis in this species and discusses the potential factors influenc-

Received 25 January 1989; accepted 3 April 1989.


10 cm
1


SP
W
C
30 µm
2


5 µm
SP
IS
3

SPG
PHS
HS
M
BL
SPG
CP
CT
C
4 µm
4

ing the evolution of these unusual gametes. This investigation represents only the third study of gamete ultrastructure in a deep-sea organism. The ultrastructure of sperm development in the vestimentiferan, *Riftia pachyptila,* was previously described from specimens collected near hydrothermal vent communities using the manned submersible Alvin (Gardiner and Jones, 1985). Recently, aberrant sperm were reported in *Xyloplax,* a deep sea representative of the new echinoderm class, Concentricycloidea (Healy *et al.,* 1988).

Materials and Methods

Six live, sexually mature specimens of *Phrissocystis multispina* (four male and two female) were collected off Kailua-Kona, Hawaii, in July 1988 at depths ranging from 875 m to 1920 m using the Pisces V submersible (Fig. 1). Specimens were dissected on board ship shortly after collection and testes were removed for immediate fixation. Whole testes were fixed for 1 h in a primary fixative (2.5% glutaraldehyde in 0.2 *M* sodium cacodylate, 0.1 *M* NaCl, and 0.35 *M* sucrose at 4°C) and then washed in three changes of 0.2 *M* sodium cacodylate, 0.3 *M* NaCl, and 0.35 *M* sucrose. Tissue was then postfixed in 1% OsO_4 in 0.2 *M* sodium cacodylate, 0.3 *M* NaCl, and 0.35 *M* sucrose at room temperature. Tissues were then dehydrated in ascending concentrations of ethanol, transferred through two changes of propylene oxide, and embedded in Epon. Thick sections were cut on a Porter-Blum MT2-B ultramicrotome using a diamond knife, mounted on slides, stained with Richardson's stain, and photographed with a Zeiss WL research compound microscope. Thin sections were cut with a diamond knife and stained with alcoholic saturated uranyl acetate and aqueous lead citrate for 10 min each, then examined in a Zeiss EM9S-2 transmission electron microscope.

Dried specimens of *Phrissocystis multispina* have been deposited with the U. S. National Museum, Smithsonian Institution (USNM # E 37821).

Results

Structure of the testis wall

The testes are arborescent structures suspended by folds of the perivisceral epithelium from the interambulacral plates in the apical half of the perivisceral coelom. A cross section through a single acinus of the testis reveals a thin, apparently unflagellated outer perivisceral epithelium, followed by a thick (3.5–4 μm) collagenous connective tissue layer, a narrow perihemal sinus lined by a thin basal lamina and containing prominent muscle cells, a hemal sinus about 2 μm in width, and the testis lumen lined by a germinal epithelium (Fig. 4). The hemal sinus periodically evaginates into the testis lumen creating columns of developing sperm cells (Fig. 2). Elongated interstitial cells (Fig. 3) are often observed in association with developing germ cells and contain a variety of spherical electron-dense granules and numerous, parallel microtubules arranged in the long axis of the cell (Fig. 3, insert).

Spermatogonia and spermatocytes

We observed two morphological types of sperm in four male specimens of *Phrissocystis multispina.* The two types appear in approximately equal proportions, based on squashes of fresh testes and examination of histological sections. Due to extensive injury to specimens during submersible collection, we were unable to observe spawning in intact animals. One spermatozoan type is "typical" for echinoids while the second is bipolar-tailed and "atypical" in morphology. Limited observations of living sperm indicated that both types were motile but sluggish swimmers. Various terms have been suggested to describe polymorphic sperm based on differences in nuclear chromatin (Meves, 1903; Melone *et al.,* 1980; Buckland-Nicks *et al.,* 1982). However, the two types of sperm observed in *P. multispina* appear to have identical nuclear morphology. To avoid the proliferation of new terminology, we have adopted the terms "euspermatozoon" for the typical sperm and "paraspermatozoon" (para: near, close) for the atypical sperm as proposed by Healy and Jamieson (1981). Differentiation of these sperm cannot be distinguished until the early spermatid stage so earlier development will be described for both simultaneously. Spermiogenesis will be described separately for each sperm type.

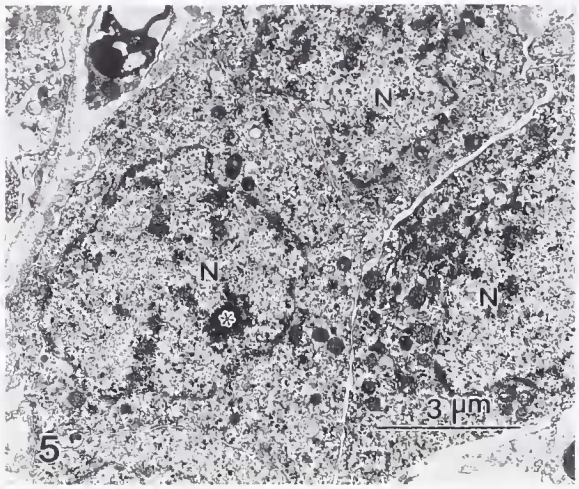
Examination of the germinal epithelium reveals the presence of spermatogonia and spermatocytes, while the

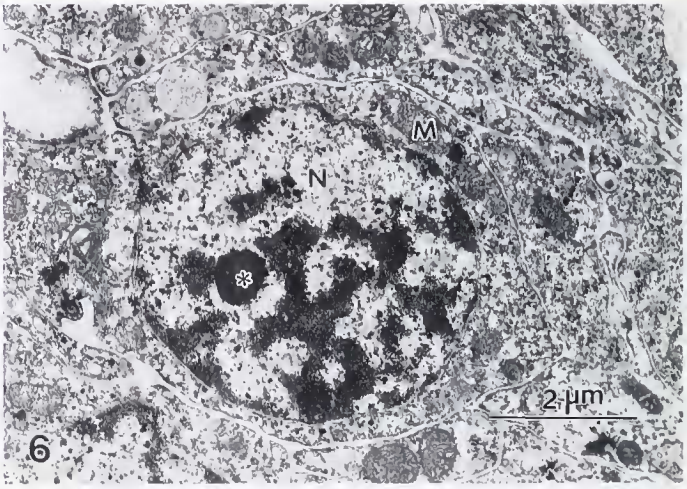
Figure 1. Adult specimen of *Phrissocystis multispina* photographed from the Pisces V submersible at a depth of 1920 m off Kailua-Kona, Hawaii.

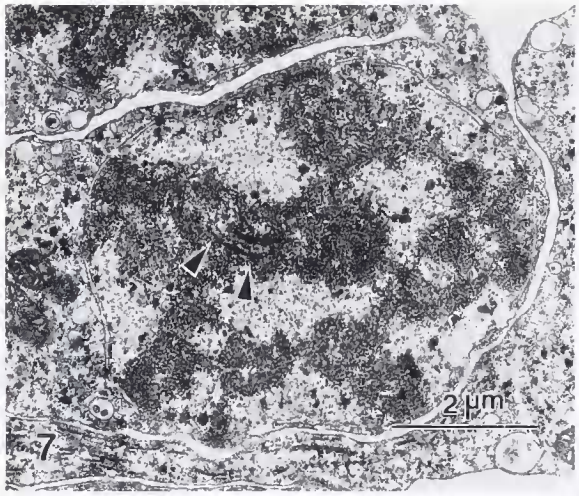
Figure 2. Light microscopic transverse section through the testis wall. Arrows indicate invagination of hemal sinus into testis lumen. C, coelom; SP, sperm cells; W, wall of testis.

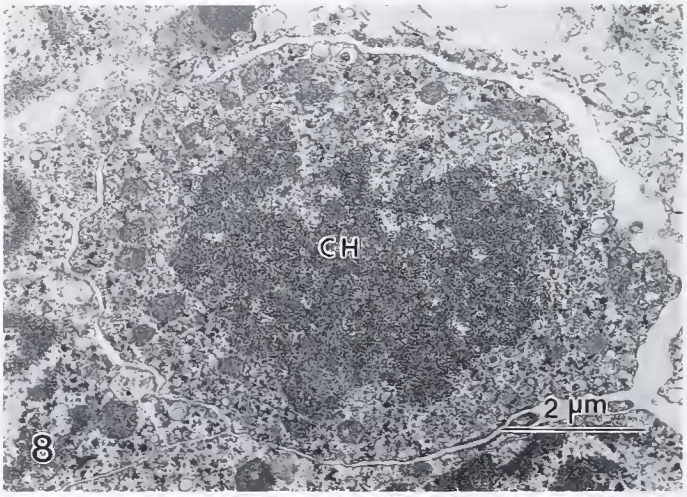
Figure 3. Interstitial cell (IS) containing electron dense inclusions (*) surrounded by developing sperm cells (SP). Insert: higher magnification of interstitial cell cytoplasm showing parallel arrays of microtubules.

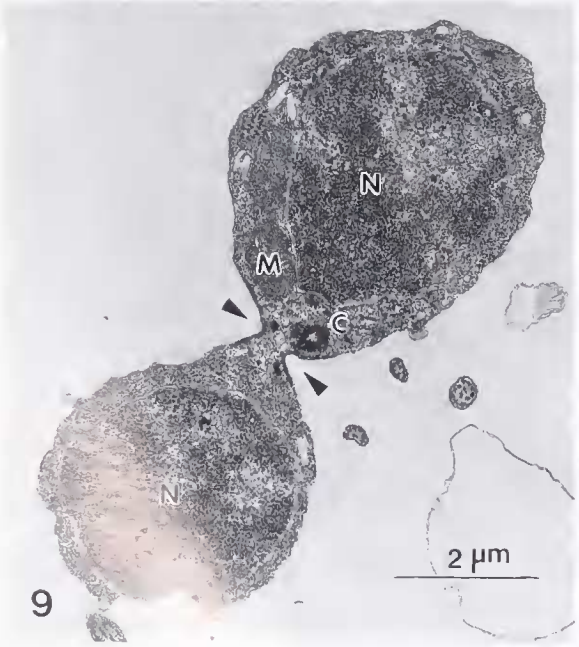
Figure 4. Transverse section through the testis wall showing the outer coelomic peritoneum (CP); connective tissue layer (CT); large muscles cells (M) within the perihemal sinus (PHS); the hemal sinus (HS) lined by a basal lamina (BL) and adjacent to a germinal epithelium containing spermatogonia (SPG) and spermatocytes (SPC). C, coelom.

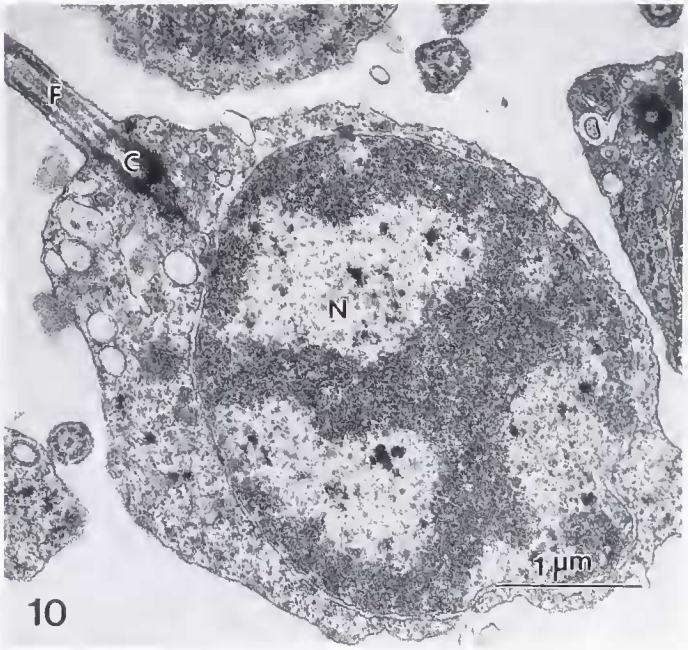

N
N
N
3 µm
5


M
N
2 µm
6


2 µm
7


CH
2 µm
8


N
M
C
N
2 µm
9


F
C
N
1 µm
10

lumen of the testis contains mostly spermatids and mature sperm. Spermatogonia lie closest to the hemal sinus (Fig. 4) and are the largest and least abundant type of germ cell (Fig. 5). They are roughly spherical in shape and contain a large (7.0–8.0 μm) nucleus, a few circular profiles of mitochondria, and numerous free ribosomes. The nucleus contains a fine granular chromatin and usually a small single nucleolus. Spermatocytes in the preleptotene/leptotene stage of first meiotic prophase are the most common germ cell and are generally spherical cells with smaller (5.0–6.0 μm) nuclei each containing a prominent nucleolus and scattered patches of electron-dense heterochromatin (Fig. 6). Occasionally, a spermatocyte in the zygotene/pachytene stage of first meiotic metaphase is observed with characteristic synaptonemal complexes (Fig. 7). Spermatocytes in the process of mitotic division are relatively common (Fig. 8).

Spermiogenesis—euspermatozoan

Spermatids are small spherical cells with round nuclei about 2.5–3 μm in diameter that are interconnected by intercellular bridges (Fig. 9). In thin sections, as many as three spermatids have been observed in cytoplasmic continuity. Bridges are characterized by electron-dense plaques along the inner plasmalemma. Spermatid nuclei have regions of electron-opaque chromatin surrounded by a finely condensed background. Organelles are sparse and include a few small mitochondria and a pair of centrioles that lie near the intercellular bridges, thus initiating a cytoplasmic polarization (Fig. 9). The distal centriole forms a single flagellum that projects from the future caudal region of the cell (Fig. 10).

Spermatids break their intercellular connections and float freely in the testis lumen when the nuclear chromatin becomes equally distributed throughout the nucleus and granular in texture (Fig. 11). A shallow centriolar fossa appears at the base of the nucleus and an electron-dense plaque forms along the inner surface of the nuclear envelope. The proximal centriole lies perpendicular to the long axis of the cell and bears a fibrous appendage that extends from its proximal surface into the centriolar fossa (Figs. 11, 12). The distal centriole and the spermatid flagellum lie posterior to the proximal centriole.

As differentiation continues, the entire cell and its nucleus elongate (Fig. 12). The mitochondria have now presumably fused because only a single large one remains. It is positioned in the future middlepiece region of the spermatid and surrounds both centrioles (Fig. 12). A small proacrosomal granule appears abruptly at the future apical pole of the spermatid in close association with the nucleus (Fig. 13). It consists of a small sac containing a fine granular product, located between the outer nuclear envelope and the cell plasmalemma. A Golgi complex was never observed in developing sperm cells at this or any other stage despite examination of numerous semi-serial sections. However, serial sectioning of sperm stages was not undertaken.

Further differentiation results in a mature spermatozoon (Figs. 14, 15) with a head length (middlepiece, nucleus, and acrosome) of about 4.5 μm. The nucleus is about 4.0 μm in length and 2.0 μm in width, and bears a small, cap-like acrosomal vesicle measuring about 300 nm in length and 275 nm in width. The vesicle is subspherical with a flattened basal surface (Fig. 17). It contains a fine granular product and the inner surface of the posterior acrosomal membrane bears an electron-dense plaque. A small subacrosomal fossa forms a depression at the anterior tip of the nucleus and contains fine, reticular periacrosomal material. The middlepiece is symmetrical with centrally placed centrioles at right angles to each other surrounded by a single, circular mitochondrion (Fig. 16). The distal centriole rests in a shallow centriolar fossa at the base of the nucleus (Fig. 14). The flagellum exhibits the 9 + 2 pattern (Fig. 14, insert).

Spermiogenesis—paraspermatozoan

Due to the limited amount of material available for ultrastructural study, we were unable to conclusively elucidate all stages in the development of this sperm type, particularly with respect to the differentiation of the flagellum.

Early spermatids resemble those of the euspermatozoa in most respects except for the appearance of two axonemes, one of which remains intracellular. Only two centrioles were ever observed, and to avoid confusion, the centriole producing the primary flagellum used in propulsion will be designated the distal centriole. The proximal centriole forms a secondary axoneme which remains intracellular and closely associated with the nucleus throughout spermiogenesis.

Figure 5. Three spermatogonia with large, irregularly shaped nuclei (N) and small nucleolus (*).

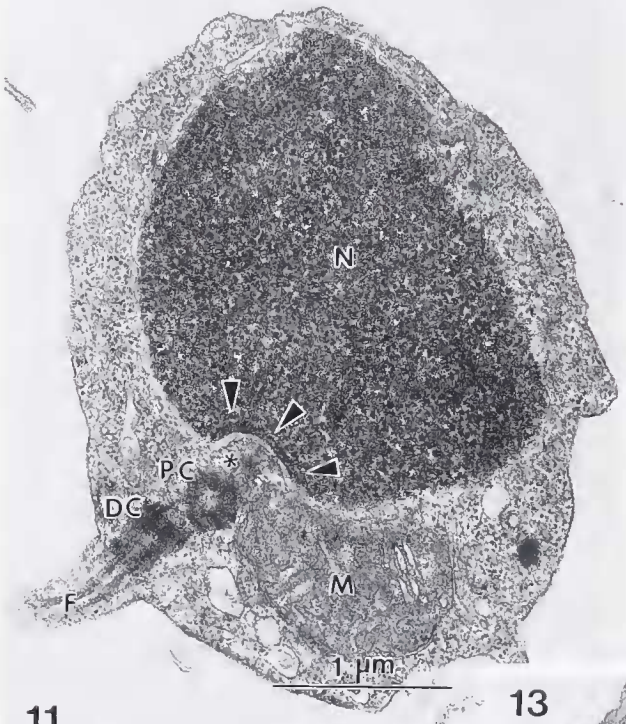
Figure 6. Spermatocyte with large circular nucleus containing a small nucleolus (*). M, mitochondrion.

Figure 7. Spermatocyte nucleus containing synatonemal complex (arrows).

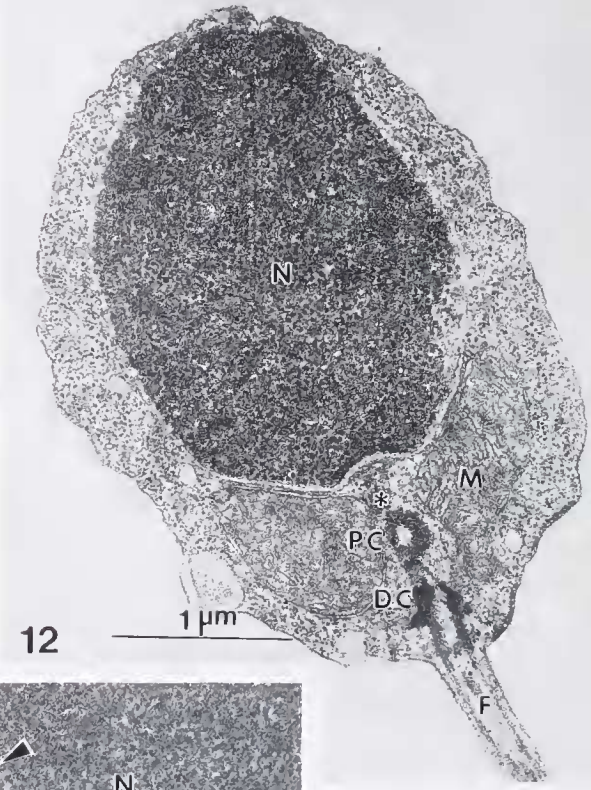
Figure 8. Spermatocyte in process of mitotic division. Note dispersed chromatin (CH).

Figure 9. Two spermatids connected by intercellular bridge (between arrows). N, nucleus; C, centriole; M, mitochondrion.

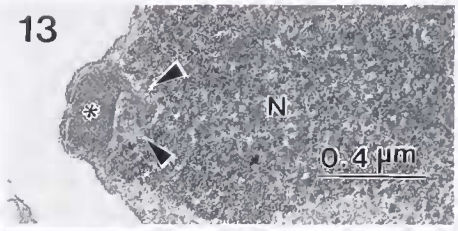
Figure 10. Spermatid with large nucleus (N), posterior flagellum (F) and associated centriole (C).



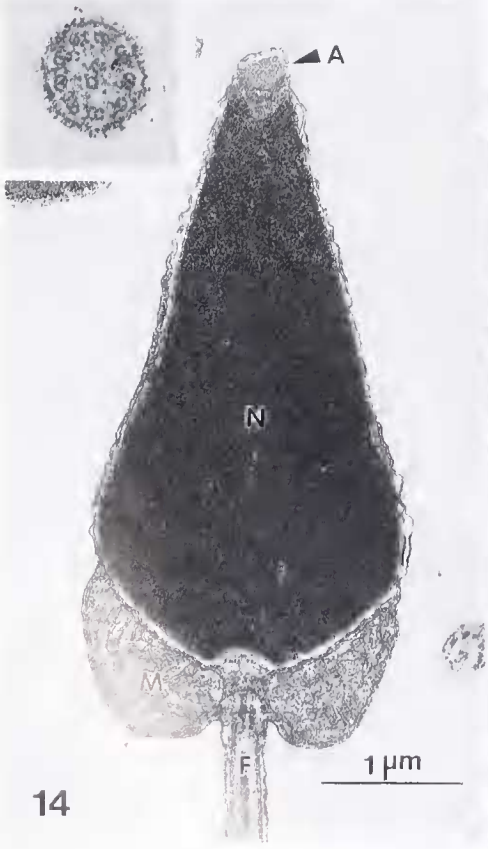
11



12

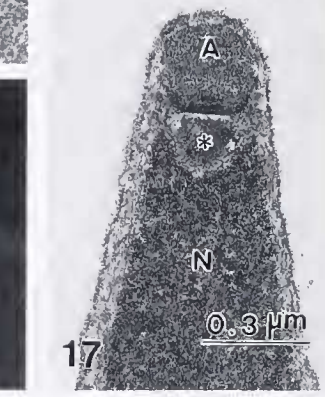


13

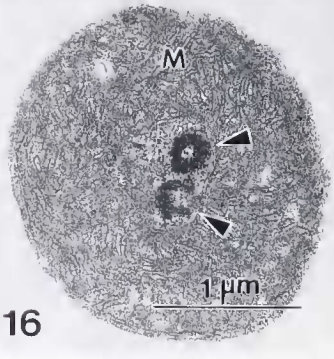


14

15



17



16

The primary flagellum projects from the spermatid near the intercellular bridge (Fig. 18) while a secondary axoneme remains intracellular (Figs. 19, 20). The proximal centriole bears a fibrous appendage that extends into the shallow centriolar fossa, similar to that described in the euspermatozoa (Figs. 19, 21, 22).

The spermatid nucleus begins a process of nuclear reduction beginning with the formation of a redundant nuclear envelope that divides the nucleus into two closely apposed, approximately equal-sized halves (Fig. 23). The mitochondria congregate at the base of one nucleus (Fig. 23) and both nuclei begin a process of chromatin condensation (Fig. 24). A small proacrosomal vesicle appears near the future apical region of the cell (Fig. 24, insert). The process of chromatin condensation continues in only one nucleus and a shallow centriolar fossa forms at its base (Fig. 25). The second nucleus gradually condenses into an electron-dense inclusion that is cast off as superfluous chromatin (Figs. 26, 27, 31, 36).

The remaining spermatid nucleus elongates and tapers anteriorly. Many nuclei contain a number of nuclear vacuoles (Fig. 30). The proximal centriole and its associated axoneme extends anteriorly and lateral to the nucleus (Fig. 28) with the centriole eventually coming to rest within the centriolar fossa at the base of the nucleus (Fig. 30). Microtubular components of the axoneme formed by the distal centriole separate, with a portion circling the nucleus and the remainder contributing to the posterior flagellum (Figs. 26, 27, 29). The anteriorly directed component of the axoneme extends well beyond the nucleus (Fig. 31), reaching a length of about 75 μm. The fibrous appendage associated with the proximal centriole disappears at this stage.

The mature paraspermatozoon bears an apical acrosome similar to that of the euspermatozoon (Figs. 31–33). A single primary flagellum lies parallel to the long axis of the nucleus and extends about equidistant both anteriorly and posteriorly to create a bipolar-tailed spermatozoon (Figs. 35, 40, 41). This primary flagellum appears to originate from the distal centriole but is joined just anterior to the acrosome by a secondary axoneme originating from the proximal centriole at the base of the nucleus (Fig. 33). Therefore, two 9 + 2 axonemes originating from separate centrioles lie on opposite sides of the nucleus (Fig. 39) and fuse into a single flagellum anteriorly (Fig. 33 and insert). Longitudinal and transverse sections of the distal region of this flagellum show that it is variable in thickness and is composed of disorganized microtubular doublets that taper near the terminal region (Figs. 34, 35, insert). The distal and proximal centrioles lie nearly parallel to each other within the centriolar fossa, surrounded by a single circular mitochondrion (Figs. 37, 38). Microtubules associated with the distal centriole fuse with the primary flagellum soon after emerging from the posterior region of the cell (Figs. 35–37). Scanning electron micrographs of the mature paraspermatozoa show the bipolar-tailed cell and the lateral displacement of the posterior-directed flagellum which creates a rotational asymmetry (Figs. 40–41). The anterior and posterior portions of the flagellum each extend about 75 μm from the nucleus.

Discussion

Sperm dimorphism has not been reported previously in echinoderms although it exists in a number of other invertebrate groups (see reviews by Fain-Maurel, 1966; Roosen-Runge, 1973, 1977; Healy and Jamieson, 1981; Buckland-Nicks *et al.*, 1982; Jamieson, 1987). The possible functions of paraspermatozoa have been discussed by several authors (Nishiwaki, 1964; Fain-Maurel, 1966; Baccetti and Afzelius, 1976; Healy and Jamieson, 1981) and include the transporting of euspermatozoa from one animal to another (see Nishiwaki, 1964), the prevention of premature euspermatozoan dispersal by entanglement in the multiple tails of the paraspermatozoa (Woodard, 1940), and the nutrition and/or stimulation of euspermatozoa by paraspermatozoan breakdown products (see Buckland-Nicks and Chia, 1977). Sperm polymorphism is believed to be under genetic control with environmental factors (Ansley, 1958; Fain-Maurel, 1966) and hormonal control (Buckland-Nicks *et al.*, 1982) playing a secondary role.

Figure 11. Spermatid with granular chromatin in nucleus (N), electron-dense layer present on inner nuclear envelope (arrows) where centriolar fossa is forming. Note appendage (*) projecting from proximal centriole (PC) into centriolar depression. DC, distal centriole; F, flagellum; M, mitochondrion.

Figure 12. Spermatid with elongating nucleus (N) and single mitochondrion (M) surrounding distal (DC) and proximal (PC) centrioles. Note appendage projecting from proximal centriole (*). F, flagellum.

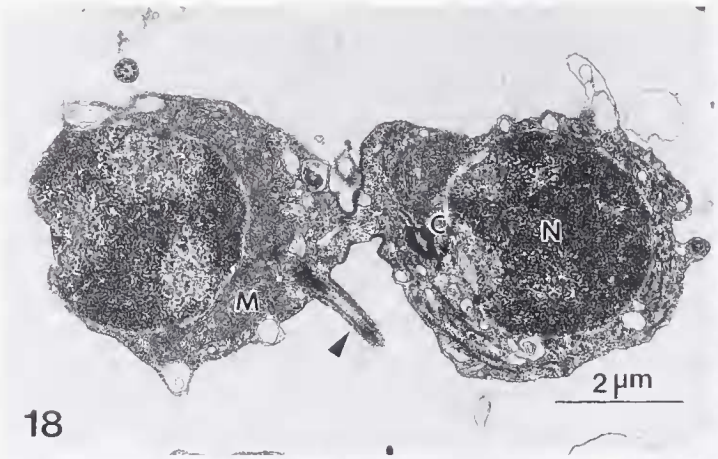
Figure 13. Proacrosomal granule (*) and differentiating subacrosomal fossa (arrows) closely associated with spermatid nucleus (N).

Figure 14. Mature euspermatozoon. A, acrosome; N, nucleus; M, mitochondrion; F, flagellum. Insert: cross section through flagellum showing 9 + 2 pattern.

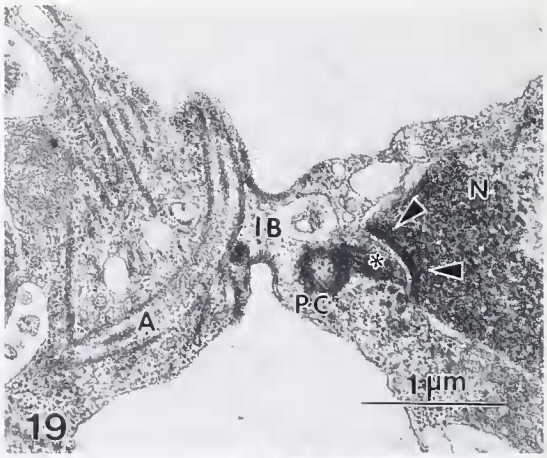
Figure 15. Scanning electron micrograph of euspermatozoon.

Figure 16. Cross section through middlepiece showing circular mitochondrion (M) and adjacent centrioles (arrows).

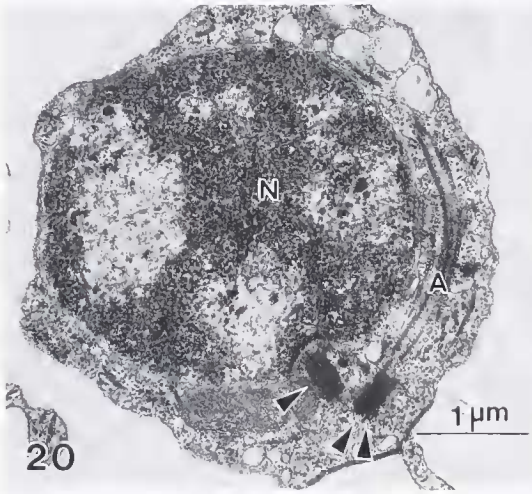
Figure 17. Acrosome (A), subacrosomal fossa (*) and nucleus (N) of mature euspermatozoon.



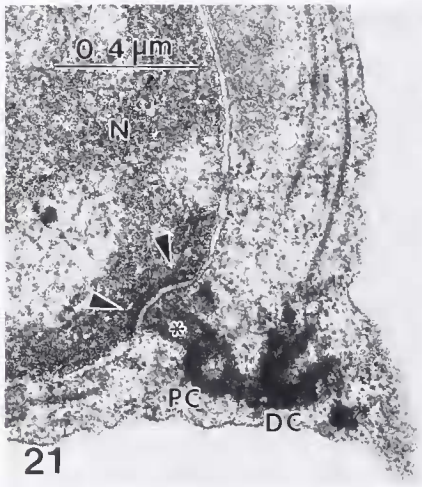
18



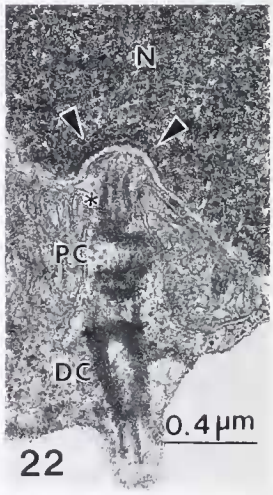
19



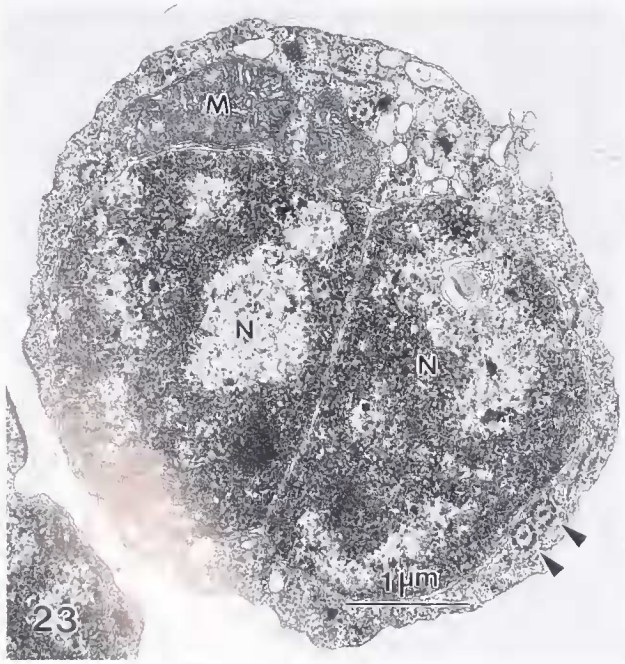
20



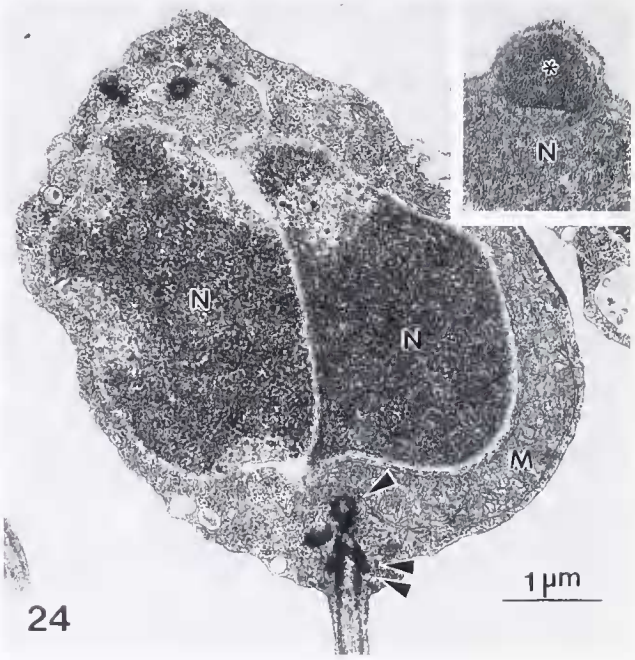
21



22



23



24

We were unable to observe spawning in *Phrissocystis multispina* so it is unclear if both the euspermatozoa and paraspermatozoa are actually released under natural conditions. However, we believe it is reasonable to assume that they are, since they were present in approximately equal proportions in the testes of each of the four mature males examined. We do not know the function of the paraspermatozoon because we were unable to perform fertilization experiments. However, the selective pressures responsible for the evolution of sperm dimorphism in this species could relate indirectly to population densities. Abyssal and bathyal echinoderm population densities are generally much lower than those of shallow water echinoderms (Pawson, 1982) and our limited submersible observations of *P. multispina* populations in Hawaii confirmed this generalization. Some mechanism is undoubtedly needed to ensure successful fertilization of eggs in widely dispersed populations. Several mechanisms could facilitate fertilization including aggregation during the breeding season, reproductive synchrony, spermatophore production, copulation, hermaphroditism, self-fertilization, and parthenogenesis. There is no direct evidence that any of these mechanisms are used by *P. multispina*.

Shallow-water echinoids aggregate during spawning seasons (Boolootian, 1966), but it is generally unclear if this results from attraction to conspecifics for purposes of reproduction or from some other cause (Pearse and Arch, 1969). However, pairing for the apparent purpose of spawning has been reported recently (Young, Tyler and Cameron, in prep.) in the deep-sea echinoids, *Stylocidaris lineata* and *Cidaris blakei*, based on *in situ* submersible observations and histological examination of the gonads.

In the absence of spermatophore production or copulation, broadcast spawning of sperm could represent a high-risk fertilization mechanism in *P. multispina* unless there are means to retard sperm dilution or otherwise enhance the probability of fertilization. Pennington (1985) demonstrated with laboratory and field experiments with *Stronglyocentrotus drobachiensis*, that freely spawned sperm are rapidly diluted over short distances resulting in low fertilization rates. Similar results have been obtained for *Diadema antillarum* (Levitan, 1988).

In limited laboratory observations of live and fixed sperm samples from *P. multispina*, we noted that sperm clumping readily occurred due to the entanglement of flagellae. Woodard (1940) conducted experiments with live sperm from the fresh water prosobranch, *Goniobasis*, and convincingly demonstrated that the euspermatozoa became entangled in the multiple tails of the paraspermatozoa, which caused clumping and prevented their wide dispersal. A similar function could be attributed to the bipolar-tailed paraspermatozoa of *P. multispina* as a means of reducing euspermatozoan diffusion during a spawning event.

The dimorphic sperm of *Phrissocystis multispina* are different from those reported in many invertebrates in that both types have acrosomes and similar nuclear morphology. In other species, it is common for the atypical spermatozoon to have greater or lesser amounts of nuclear chromatin or no nucleus at all (Healy and Jamieson, 1981) although in some instances both sperm types appear to have the full haploid chromosome compliment (Jamieson, 1987). Many authors conclude that parasperm are non-fertilizing and therefore do not contribute to the zygote genome. This is undoubtedly the case in those sperm lacking acrosomes, but little is known about the behavior of parasperm in the presence of eggs in any species (Healy and Jamieson, 1981).

The paraspermatozoa of some molluscs have no acrosomes (Yasuzumi *et al.*, 1967) and in others, the acrosomal-like structure observed in these sperm have not been proven to be of Golgi origin (Healy and Jamieson, 1981). Although we did not observe Golgi activity during the development of either sperm type in *P. multispina*, we

Figure 18. Interconnected spermatids of paraspermatozoon. N, nucleus; C, centriole; M, mitochondrion; Arrow indicates flagellum.

Figure 19. Intercellular bridge (IB) connecting two spermatids. Note early formation of centriolar fossa (arrows), fibrous appendage extending from the proximal centriole (PC), and intracellular axoneme (A). N, nucleus.

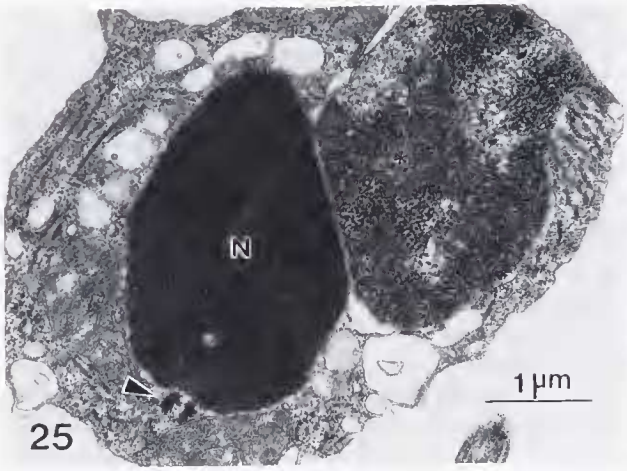
Figure 20. Early spermatid containing proximal centriole (double arrows) with associated intracellular axoneme (A), distal centriole (single arrow) and nucleus (N).

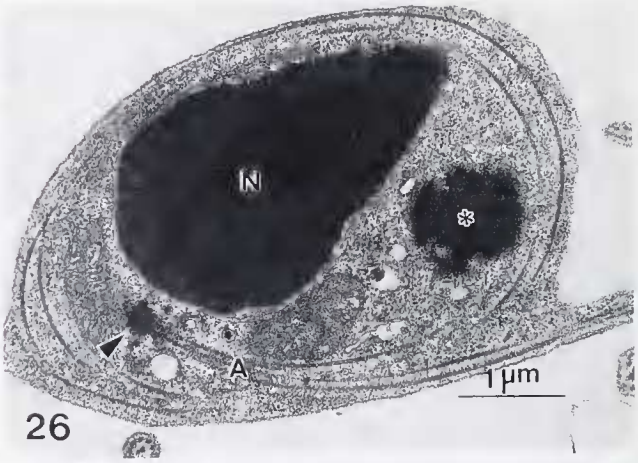
Figure 21. Proximal (PC) and distal (DC) centrioles showing fibrous appendage (*) extending from proximal centriole into centriolar fossa (arrows). N, nucleus.

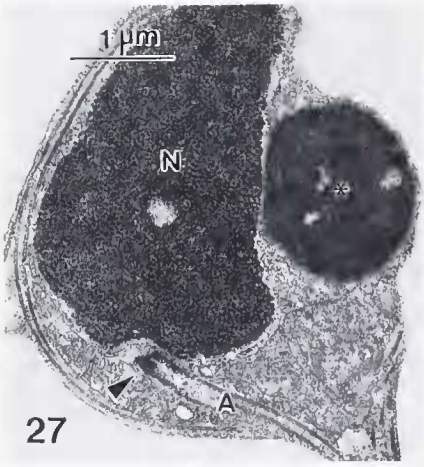
Figure 22. Fibrous appendage (*) projecting into centriolar fossa (arrows) from proximal centriole (PC). DC, distal centriole; N, nucleus.

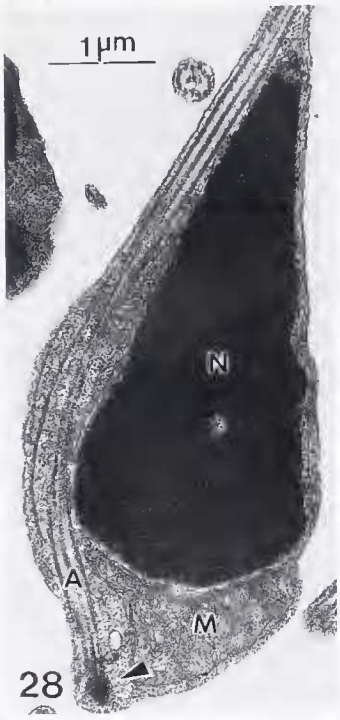
Figure 23. Spermatid with two nuclei (N). M, mitochondrion. Arrows indicate cross sections through intracellular axoneme.

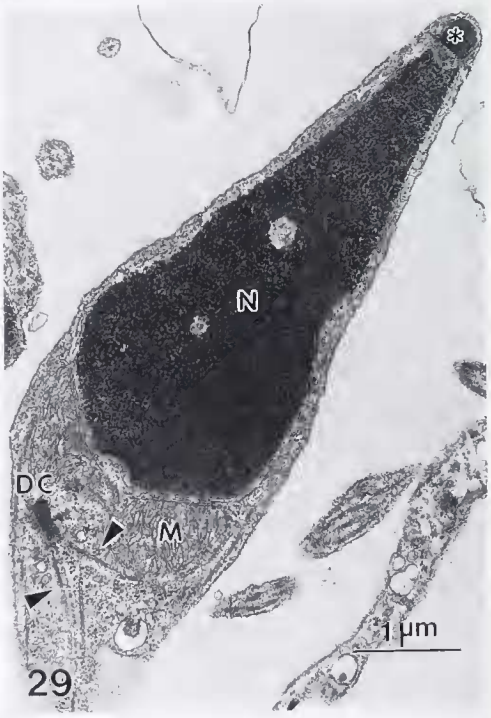
Figure 24. Two nuclei with more highly condensed chromatin (N). Proximal (single arrow) and distal (double arrows) centrioles are located at base of cell. M, mitochondrion. Insert: Proacrosomal granule (*) adjacent to nucleus (N).

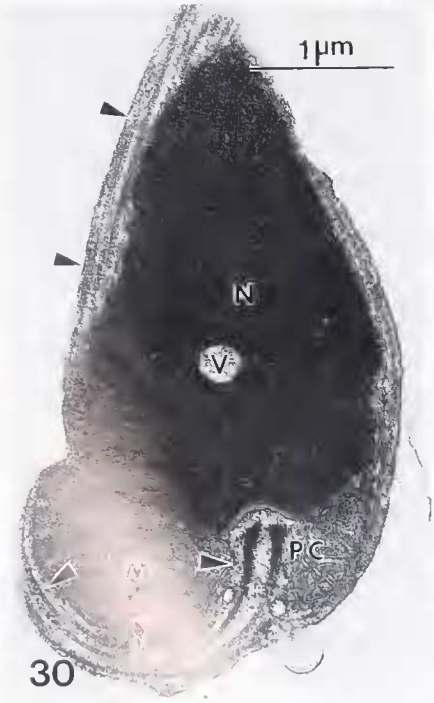

N
1 µm
25

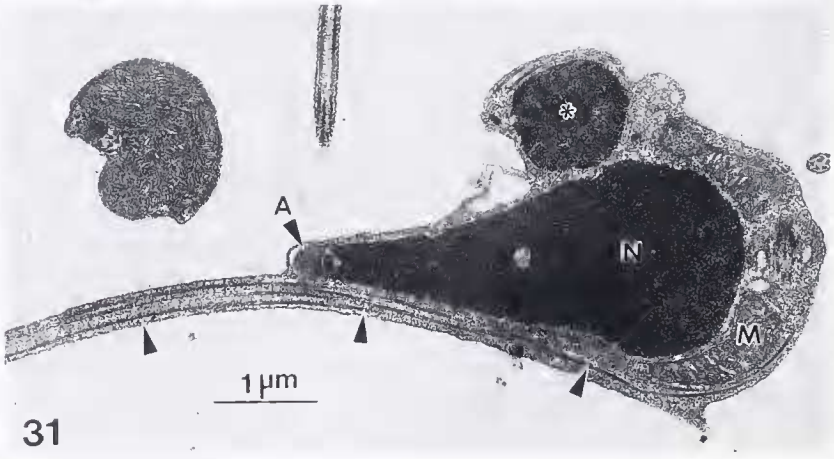

N
A
1 µm
26


1 µm
N
A
27


1 µm
N
A
M
28


N
DC
M
1 µm
29


1 µm
N
V
PC
30


A
N
M
1 µm
31

have chosen to use the term "acrosome" because this structure is morphologically consistent with the acrosomes described in detail in the sperm of numerous echinoid species (Chia and Bickell, 1983). To our knowledge, acrosomogenesis in all echinoderms is associated with Golgi complex activity, so the origin of the acrosome in *P. multispina* sperm is problematical. Most, but not all, metazoan sperm possess acrosomes and they are typically derived from the Golgi complex (Baccetti and Afzelius, 1976). In rare instances, acrosome-like structures are formed without the apparent involvement of the Golgi complex as, for instance, in some gastropod sperm (Garreau de Loubresse, 1971; Eckelbarger and Eyster, 1981). Careful serial sectioning of sperm stages would resolve this question but, until then we will assume that the acrosome observed in the sperm of *P. multispina* sperm has a Golgi origin.

Bipolar-tailed animal sperm are rare, having been reported only from the paraspermatozoa of some archaeogastropod molluscs of the family Neritidae (Nishiwashi, 1964; Tochimoto, 1967). These were light microscopical observations so ultrastructural comparisons to the sperm of *P. multispina* cannot be made. The existence of a bipolar-tailed spermatozoon in *Phrissocystis multispina* is notable because echinoderm sperm are among the best known among invertebrates and are characteristically conservative in morphology (Field, 1895; Tyler and Tyler, 1966; Chia *et al.*, 1975; Eckelbarger *et al.*, in press). Sperm morphology has been described for nearly 130 echinoderm species from shallow water, bathyal and deep sea habitats (Eckelbarger *et al.*, in press). The majority of echinoderms release their gametes directly into seawater and their sperm are largely categorized as "primitive" using the definition of Franzen (1956, 1970). Asteroid and ophiuroid sperm are typically spherical, as are those of crinoids with two known exceptions, the tabloid-headed sperm of *Isometra vivipara* (Holland, 1976a), and the conical-headed sperm of *Antedon bifida* (Holland, 1976b). Echinoid sperm heads are usually conical while holothuroid sperm heads are spherical with two known exceptions, the cylindrical-headed sperm of *Cucumaria lubrica* (Atwood and Chia, 1974) and the tabloid-headed sperm of *Cucumaria pseudocurata* (Atwood, 1975). Recently, however, an unusual spermatozoon was described from the bathyal echinoid, *Aspidodiadema jacobyi*, in which the head contains a thin, tapering, sickle-shaped nucleus reaching 27 μm in length (Eckelbarger *et al.*, in press).

Recent ultrastructural studies of sperm were reported from museum specimens of *Xyloplax turnerae*, a deep-water representative of the newly erected class of echinoderms, the Concentricycloidea (Healy *et al.*, 1988). The sperm are highly aberrant relative to other echinoderms, consisting of an elongate, internally segmented acrosome; a long tapering nucleus; a single flagellum attached via a centriolar rootlet to the anterior portion of the nucleus; and a single elongate mitochondrion located posterior to the nucleus. The authors concluded that the numerous specialized features of this sperm suggest substantial genetic differences between the Concentricycloidea and other echinoderms and offers strong support for the recognition of the new class proposed by Baker *et al.* (1986) and Rowe *et al.* (1988). The authors dismissed any obligate correlation between modified sperm morphology and abyssal existence because a parallel ultrastructural study of the abyssal asteroid *Caymanostella* sp. from the same habitat showed it to be typical of other asteroid sperm.

However, several recent findings suggest that deep-sea echinoderm populations have undergone a series of novel reproductive adaptations in response to selective pressures apparently unique to deep-sea habitats. A structurally new type of ovary has been described from the bathyal holothuroid *Holothuria occidentalis*, in which podocytes, cells usually involved in fluid ultrafiltration in excretory organs, are intimately associated with developing oocytes (Eckelbarger *et al.*, 1988). Furthermore, an examination of the sperm from 23 species of bathyal echinoderms revealed a significant positive correlation between head length (acrosome, nucleus, and

Figure 25. Two nuclei within a spermatid showing the highly condensed primary nucleus (N) and the degenerating secondary nucleus (*). Arrow indicates centriole.

Figure 26. Spermatid containing elongating nucleus (N), degenerating nucleus (*) and distal centriole (arrow) with associated axoneme (A).

Figure 27. Distal centriole (arrow) and associated axoneme (A) in spermatid. N, nucleus; *, degenerating nucleus.

Figure 28. Distal centriole (arrow) and associated axoneme (A) lying adjacent to long axis of nucleus (N). M, mitochondrion.

Figure 29. Distal centriole (DC) with splayed axoneme (arrows). M, mitochondrion; N, nucleus; *, acrosome.

Figure 30. Proximal centriole (PC) and anteriorly directed axoneme (arrows). M, mitochondrion; N, nucleus.

Figure 31. Advanced spermatid bearing anteriorly directed portion of primary axoneme (arrows), terminal acrosome (A) and degenerating nucleus (*). M, mitochondrion; N, nucleus.

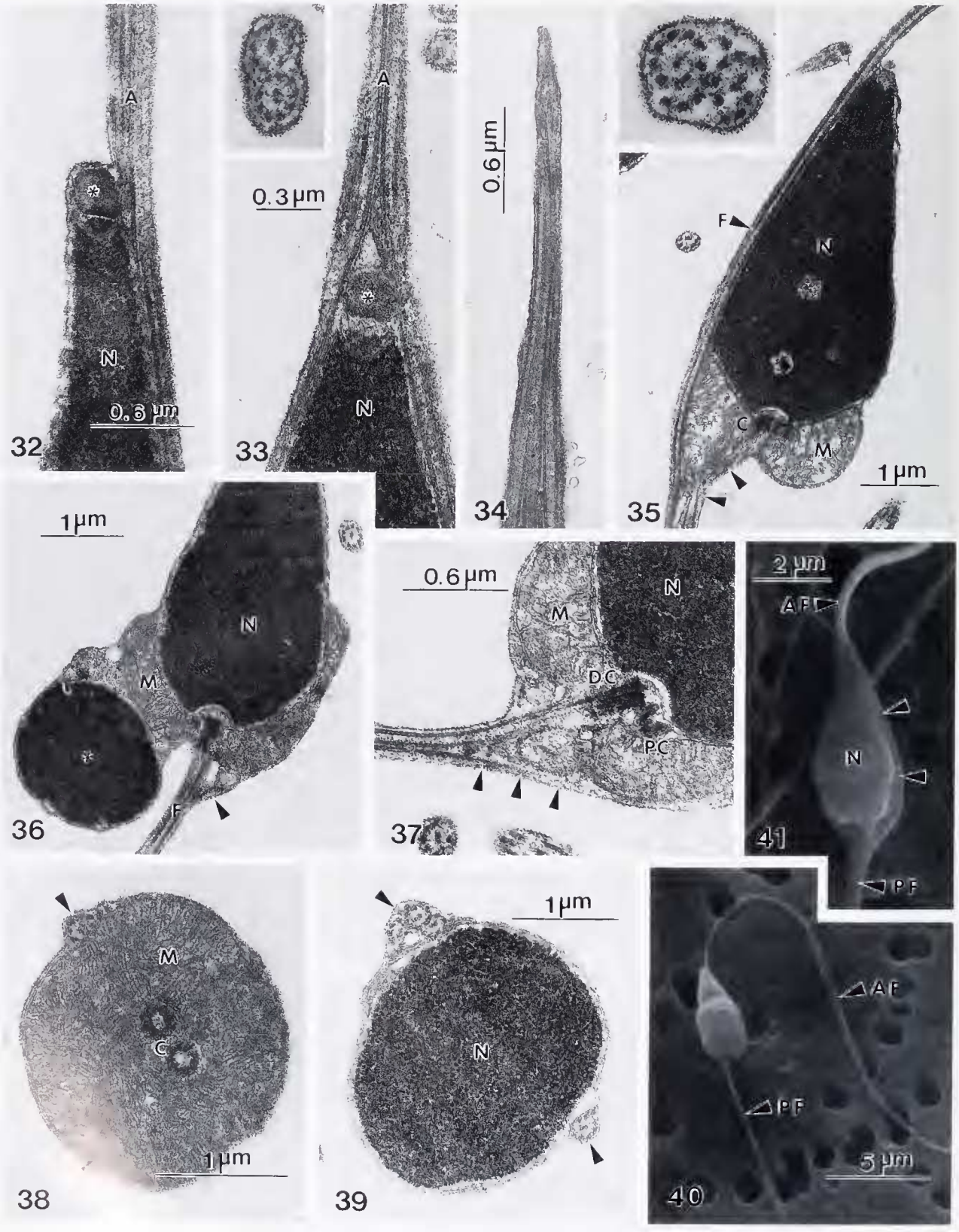


Figure 32. Apical region of paraspermatozoon nucleus (N) showing acrosome (*) and anteriorly directed axoneme (A).

Figure 33. Apical region of nucleus (N) showing acrosome (*) and lateral components of two lateral

middlepiece) and depth distribution among echinoids. Included in these observations is the aberrant sperm from the bathyal echinoid *Aspidodiadema jacobyi* (Eckelbarger *et al.*, in press). Finally, a unique type of larval development was described in *A. jacobyi* in which small (90 μm) eggs spend sequential portions of their planktonic development in lecithotrophic and planktotrophic phases (Young *et al.*, in prep.).

The unusual morphological specializations reported in deep-water echinoderm sperm can largely be attributed to alterations in fertilization biology. Franzen (1956) noted that structural modifications of sperm often are associated with altered demands on the mobility of the sperm, particularly when they must swim through a viscous medium. The evolution of derived sperm reported in the externally brooding holothuroids *Cucumaria pseudocurata* (Atwood, 1975) and *C. lubrica* (Atwood, 1974; Atwood and Chia, 1974) has been attributed to the presence of viscous mucus surrounding the egg mass through which the sperm must pass (Atwood and Chia, 1974; Atwood, 1975). In *Aspidodiadema jacobyi*, eggs are released in a mucus mass through which presumably the modified sperm must pass (Eckelbarger *et al.*, in press).

In the Concentricycloids *Xyloplax turnerae* and *X. medusiformis*, it is believed that sperm transfer involves copulation between male and female using modified spinelets of the male (Rowe *et al.*, 1988). The existence of highly aberrant spermatozoa in these species correlates nicely with the mechanism of fertilization because modification of sperm morphology is directly related to the evolution of internal fertilization (Franzen, 1956, 1966; Afzelius, 1977). Egg size may also influence the length of the sperm head in some species. An analysis of sperm head length and egg size in bathyal populations of echinoids and holothuroids revealed a strong positive correlation indicating that larger eggs are fertilized by larger sperm (Eckelbarger *et al.*, in press). Franzen (1983) noted a similar correlation between the evolution of elongated sperm nuclei and large yolk-rich eggs in bivalve molluscs. However, one must be cautious in drawing correlations between sperm modifications and fertilization biology in echinoderms because in some ophiuroids, asteroids, and holothuroids, primitive sperm are not always encountered in externally fertilizing species and derived sperm are not consistently observed in species in which fertilization is internal (Chia *et al.*, 1975; Janssen, 1984; Healy *et al.*, 1988).

Despite the widespread use of echinoid sperm in modern developmental biology studies, there is a paucity of information regarding their development (Chia and Bickell, 1983). Sperm morphology is known for nearly 70 echinoid species (Chia *et al.*, 1975; Eckelbarger *et al.*, in press), but ultrastructural studies of echinoid spermatogenesis are confined to only four species (Longo and Anderson, 1969; Cruz-Landim and Beig, 1976). Many features of development of the dimorphic sperm of *Phrissocystis multispina* parallel those of other echinoids as well as other echinoderms. However, differentiation of the nucleus and flagellum are notable exceptions.

During early spermiogenesis in the paraspermatozoa of *Phrissocystis multispina*, chromatin reduction is accomplished by the formation of a redundant nuclear envelope in a manner similar to that reported in some ver-

---

axonemes which fuse into a single axoneme (A) just anterior to the acrosome. Insert: cross section through two 9 + 2 axonemes just anterior to acrosome.

Figure 34. Longitudinal section through tapering terminal region of anteriorly directed flagellum showing tapering tip.

Figure 35. Longitudinal section through paraspermatozoon showing anteriorly and posteriorly directed flagellum (F) and two adjacent centrioles (C). Arrows indicate microtubular components extending from distal centriole which join the posteriorly directed portion of the flagellum. M, mitochondrion. Insert: cross section through terminal region of anteriorly directed axoneme shown in Figure 34. Note disorganized arrangement of microtubules.

Figure 36. Spermatid with superfluous nuclear chromatin inclusion (*). Arrow indicates branching of posterior flagellum (F) as it extends anteriorly alongside the nucleus (N). M, mitochondrion.

Figure 37. Middlepiece region of paraspermatozoon showing the association of the microtubular elements of the primary flagellum (arrows) with those emanating from the distal centriole (DC). PC, proximal centriole; M, mitochondrion; N, nucleus.

Figure 38. Cross section through the middlepiece region showing the circular mitochondrion (M), the parallel centrioles (C) and the oblique section through the laterally positioned axoneme (arrow).

Figure 39. Cross section through the central nuclear region (N) showing axonemes on both sides of the nucleus (arrows).

Figure 40. Scanning electron micrograph of mature paraspermatozoon showing posteriorly (PF) and anteriorly directed (AF) components of the flagellum.

Figure 41. Scanning electron micrograph of nuclear region (N) of paraspermatozoon showing anteriorly (AF) and posteriorly directed (PF) components of flagellum. Arrows indicate primary axoneme lateral to nucleus. Compare this micrograph to TEM section shown in Figure 35.

tebrate sperm (see Yasuzumi, 1974). This results in a division of the nucleus into two approximately equal regions, one of which degenerates and is apparently cast off. During development of the apyrene carrier spermatozoon in the mollusc *Fusitriton oregonensis*, the nuclear envelope pinches off a series of lobes from the nucleus, resulting in discrete vacuoles (Buckland-Nicks *et al.*, 1982) termed "carymerites" by earlier authors (Goldschmidt, 1916). In *F. oregonensis*, the caryomerites undergo intracellular digestion while in the "atypical" sperm of other animals, nuclear remnants are excreted from the cell or converted into glycoproteins and stored in the middlepiece (see Buckland-Nicks *et al.*, 1982, for discussion).

The details of the process are not entirely clear, but the primary flagellum in the paraspermatozoon of *Phrissocystis multispina* appears to be formed by the distal centriole as in other echinoderm sperm. However, the proximal centriole, which plays no role in flagellum formation in other echinoderm sperm (Chia and Bickell, 1983), forms an intracellular, secondary axoneme which fuses with the primary flagellum. The function of this secondary axoneme is unknown, but we assume it is immotile and perhaps provides structural support to the primary flagellum during propulsion.

Acknowledgments

Submersible time was provided by the N.O.A.A. National Undersea Research Program through the Hawaii Undersea Research Laboratory. We thank the Captain and crew of the R/V Kila and the H.U.R.L. operations personnel for assistance at sea and P. A. Linley for assistance in specimen preparation for electron microscopy. Appreciation is also extended to Dr. Richard Mooi, Smithsonian Institution, for identifying specimens of *Phrissocystis multispina*. The study was supported in part by NSF grant OCE-8717922. This represents contribution number 711 of Harbor Branch Oceanographic Institution.

Literature Cited

Afzelius, B. A. 1977. Spermatozoa and spermatids of the crinoid *Antedon petasus*, with a note on primitive spermatozoa from deuterostome animals. *J. Ultrastruc. Res.* **59:** 272–281.

Ahfeld, T. E. 1977. A disparate seasonal study of reproduction of eight deep-sea macroinvertebrate species from the Northwestern Atlantic Ocean. Ph.D. dissertation, Florida State University, Tallahassee. 105 pp.

Ansley, H. R. 1958. Histones of mitosis and meiosis in *Loxa flavicollis* (Hemip[illegible]. *J. Biophys. Biochem. Cytol.* **4:** 59–62.

Atwood, D. G. 1974. [illegible] structure of spermatogonia, spermatocytes, and spermatids of [illegible] sea cucumbers *Cucumaria lubrica* and *Leptosynapta clarki* (Echinodermata: Holothuroidea). *Can. J. Zool.* **52:** 1389–1396.

Atwood, D. G. 1975. Fine structure of an elongated dorso-ventrally compressed echinoderm (Holothuroidea) spermatozoon. *J. Morphol.* **145:** 189–207.

Atwood, D. G., and F.-S. Chia. 1974. Fine structure of an unusual spermatozoon of a brooding sea cucumber, *Cucumaria lubrica*. *Can. J. Zool.* **52:** 519–523.

Baccetti, B., and B. A. Afzelius. 1976. *The Biology of the Sperm Cell.* S. Karger, New York. 254 pp.

Baker, A. N., F. W. E. Rowe, and H. E. S. Clark. 1986. A new class of Echinodermata from New Zealand. *Nature* **321:** 862–864.

Billet, D. S. M., and B. Hansen. 1982. Abyssal aggregations of *Kolga hyalina* Danielssen and Koran (Echinodermata: Holothuroidea) in the northeast Atlantic Ocean: a preliminary report. *Deep Sea Res.* **29:** 799–818.

Boolootian, R. A. 1966. Reproductive physiology. Pp. 561–614 in *Physiology of Echinodermata*, R. A. Boolootian, ed. Wiley (Interscience), New York.

Buckland-Nicks, J. A., and F.-S. Chia. 1977. On the nurse cell and the spermatozeugma in *Littorina sitkana*. *Cell Tiss. Res.* **179:** 347–356.

Buckland-Nicks, J., D. Williams, F.-S. Chia, and A. Fontaine. 1982. The fine structure of the polymorphic spermatozoa of *Fusitriton oregonensis* (Mollusca: Gastropoda), with notes on the cytochemistry of the internal secretions. *Cell Tissue Res.* **227:** 235–255.

Chia, F.-S., and L. R. Bickell. 1983. Echinodermata. Pp. 545–620 in *Reproductive Biology of Invertebrates*, Vol. II: *Spermatogenesis and Sperm Function*, K. G. and R. G. Adiyodi, eds., John Wiley & Sons, New York.

Chia, F.-S., D. G. Atwood, and B. Crawford. 1975. Comparative morphology of echinoderm sperm and possible phylogenetic implications. *Am. Zool.* **15:** 533–565.

Cruz-Landim, C., and D. Beig. 1976. Spermiogenesis in the sea urchins *Arbacia lixula* and *Echinometra lucunter* (Echinodermata). *Cytologia* **41:** 331–344.

Eckelbarger, K. J., and L. S. Eyster. 1981. An ultrastructural study of spermatogenesis in the nudibranch mollusc *Spurilla neapolitana*. *J. Morphol.* **170:** 283–299.

Eckelbarger, K. J., C. M. Young, and J. L. Cameron. In press. Modified sperm in echinoderms from the bathyal and abyssal zones of the deep sea. International Symposium Series: *23rd European Marine Biology Symposium*, P. A. Tyler and J. Ryland, eds. Olsen & Olsen, Denmark.

Eckelbarger, K. J., C. M. Young, and J. L. Cameron. 1988. Unusual gonads and bizarre gametes from deep-sea echinoderms. *Am. Zool.* **28:** 199A.

Fain-Maurel, M. A. 1966. Acquisitions recentes sur les spermatogeneses atypiques. *Ann. Biol.* **5:** 513–564.

Field, G. W. 1895. On the morphology and physiology of the echinoderm spermatozoan. *J. Morphol.* **11:** 235–270.

Franzen, A. 1956. On spermiogenesis, morphology of the spermatozoan, and biology of fertilization among invertebrates. *Zool. Bidr. Uppsala* **30:** 355–482.

Franzen, A. 1970. Phylogenetic aspects of the morphology of spermatozoa and spermiogenesis. Pp. 29–46 in *Comparative Spermatology*, B. Baccetti, ed. Academic Press, New York.

Franzen, A. 1983. Ultrastructural studies of spermatozoa in three bivalve species with notes on evolution of elongated sperm nucleus in primitive spermatozoa. *Gamete Res.* **7:** 199–214.

Gardiner, S. L., and M. L. Jones. 1985. Ultrastructure of spermiogenesis in the Vestimentiferan tube worm *Riftia pachyptila* (Pogonophora: Obturata). *Trans. Am. Microsc. Soc.* **104:** 19–44.

Garreau de Loubresse, N. 1971. Spermiogenese d'un gasteropode prosobranchi: *Nerita senegolensis;* evolution du canal intranucleaire. *J. Microsc. Paris* **12:** 425–440.

George, R. Y. and R. J. Menzies. 1967. Indication of cyclic reproductive activity in abyssal organisms. *Nature* **215:** 878.

George, R. Y., and R. J. Menzies. 1968. Further evidence for seasonal breeding cycles in deep sea. *Nature* **220:** 80–81.

Goldschmidt, R. 1916. The function of apyrene spermatozoa. *Science* **44:** 544–546.

Healy, J. M. 1988. Sperm morphology and its systematic importance in the Gastropoda. *Malcol. Rev.* **4:** 251–266.

Healy, J. M., and B. G. M. Jamieson. 1981. An ultrastructural examination of developing and mature paraspermatozoa in *Pyrazus ebeninus* (Mollusca, Gastropoda, Potamididae). *Zoomorphology* **98:** 101–119.

Healy, J. M., F. W. E. Rowe, and D. T. Anderson. 1988. Spermatozoa and spermiogenesis in *Xyloplax* (Class Concentricycloidea): a new type of spermatozoon in the Echinodermata. *Zool. Scripta* **17:** 297–310.

Holland, N. D. 1976a. Morphologically specialized sperm from the ovary of *Isometra vivipara* (Echinodermata—Crinoidea). *Acta. Zool.* **57:** 147–152.

Holland, N. D. 1976b. The fine structure of the conical-headed sperm of the crinoid *Antedon bifida*. *Experientia* **32:** 101–102.

Jamieson, B. G. M. 1987. A biological classification of sperm types with special reference to annelids and molluscs, and an example of spermiocladistics. Pp. 311–332 in *New Horizons in Sperm Cell Research*, H. Mohri, ed. Japan Sci. Soc. Press, Tokyo/Gordon and Breach Sci. Publ., New York.

Janssen, H. H. 1984. Development and ultrastructure of spermatozoa of *Archaster typicus* Mull. and Trosch. (Echinodermata, Asteroidea). *Int. J. Invert. Repro. Dev.* **7:** 333–344.

Levitan, D. R. 1988. Factors influencing fertilization success and fecundity in the sea urchin *Diadema antillarum* Philippi. *Am. Zool.* **28:** 139A.

Lightfoot, R. H., P. A. Tyler, and J. D. Gage. 1979. Seasonal reproduction in deep-sea bivalves and brittlestars. *Deep-Sea Res.* **26A:** 967–973.

Longo, F. J., and E. Anderson. 1969. Sperm differentiation in the sea urchins *Arbacia punctulata* and *Strongylocentrotus purpuratus*. *J. Ultra. Res.* **27:** 486–509.

Melone, G., C. L. L. Donin, and F. Cotelli. 1980. The paraspermatic cell (atypical spermatozoon) of Prosobranchia: a comparative ultrastructural study. *Acta Zool.* **61:** 191–201.

Meves, F. 1903. Uber oligopyrene und apyrene Spermien und uber ihre Entstehung nach Beobachtungen an *Paludina* und *Pygaera*. *Arch. Mikroskop. Anat.* **61:** 1–84.

Nishiwaki, S. 1964. Phylogenetical study on the type and of the dimorphic spermatozoa in Prosobranchia. *Sci. Rep. Tokyo Kyoiku Daigu* **11:** 237–275.

Pain, S., P. A. Tyler, and J. D. Gage. 1982a. The reproductive biology of the deep-sea asteroids *Benthopecten simplex* (Perrier), *Pectenaster filholi* Perrier, and *Pontaster tenuispinus* Dubin and Koren (Phanerozonia: Benthopectinidae) from the Rockall Trough. *J Exp. Mar. Biol. Ecol.* **65:** 195–211.

Pain, S. L., P. A. Tyler, and J. D. Gage. 1982b. The reproductive biology of *Hymenaster membranaceus* from the Rockall Trough, North-east Atlantic Ocean, with notes on *H. gennaeus*. *Mar. Biol.* **70:** 41–50.

Pawson, D. L. 1982. Deep-sea echinoderms in the Tongue of the Ocean, Bahamas Islands: a survey using the Research Submersible ALVIN. *Aust. Mus. Mem.* **16:** 129–145.

Pearse, J. S., and S. W. Arch. 1969. The aggregation behavior of *Diadema* (Echinodermata, Echinoidea). *Micronesia* **5:** 165–171.

Pennington, J. T. 1985. The ecology of fertilization of echinoid eggs: the consequences of sperm dilution, adult aggregation, and synchronous spawning. *Biol. Bull.* **169:** 417–430.

Rokop, F. J. 1974. Reproductive patterns in the deep-sea benthos. *Science* **186:** 743–745.

Rokop, F. J. 1977. Seasonal reproduction of the brachiopod *Frielia halli* and the scaphopod *Cadulus californicus* at bathyal depths in the deep sea. *Mar. Biol.* **43:** 237–246.

Roosen-Runge, E. C. 1973. Germinal-cell loss in normal metazoan spermatogenesis. *J. Reprod. Fertil.* **85:** 339–343.

Roosen-Runge, E. C. 1977. *The Process of Spermatogenesis in Animals*. Cambridge University Press, London. 145 pp.

Rowe, F. W. E., A. N. Baker and H. E. S. Clark. 1988. The morphology, development and taxonomic status of *Xyloplax* Baker, Rowe and Clark (1986) (Echinodermata: Concentricycloidea), with the description of a new species. *Proc. R. Soc. Lond. B.* **233:** 431–459.

Tochimoto, T. 1967. Comparative histochemical study on dimorphic spermatozoa of the Prosobranchia with special reference to polysaccharides. *Sci. Rep. Tokyo Kyoiku Daigu Ser. B* **13:** 75–109.

Tyler, A., and B. S. Tyler. 1966. The gametes: some procedures and properties. Pp. 639–682 in *Physiology of Echinodermata*, R. A. Boolootian, ed. Interscience, New York.

Tyler, P. A. 1988. Seasonality in the deep sea. Pp. 227–258 in *Oceanography and Marine Biology An Annual Review*, Vol. 26, M. Barnes, ed. Aberdeen University Press, Aberdeen.

Tyler, P. A., and J. D. Gage. 1982. The reproductive biology of *Ophiocantha bidentata* (Echinodermata: Ophiuroidea) from the Rockall Trough. *J. Mar. Biol. Assoc. U. K.* **62:** 45–55.

Tyler, P. A., and J. D. Gage. 1983. The reproductive biology of *Ypsilothuria talismani* (Holothuroidea: Dendrochirota) from the N.E. Atlantic. *J. Mar. Biol. Assoc. U. K.* **63:** 609–616.

Tyler, P. A., and J. D. Gage. 1984. The reproductive biology of echinothuriid and cidarid sea urchins from the deep sea (Rockall Trough, Northeast Atlantic Ocean). *Mar. Biol.* **80:** 63–74.

Tyler, P. A., A. Grant, S. A. Pain, and J. D. Gage. 1982. Is annual reproduction in deep-sea echinoderms a response to variability in their environment? *Nature* **300:** 747–750.

Woodard, T. M. 1940. The function of the apyrene spermatozoa of *Gomobasis laqueata* Say. *J. Exp. Zool.* **85:** 103–123.

Yasuzumi, G. 1974. Electron microscope studies on spermiogenesis in various animal species. Pp. 53–119 in *International Review of Cytology*, G. H. Bourne and J. F. Danielli, eds. Academic Press, New York.

Yasuzumi, G., K. J. Lee, H. Fukui, and M. Yoshida. 1967. Spermiogenesis in animals as revealed by electron microscopy. XVII. The fine structure of atypical spermatid cytoplasm of the pond snail with particular reference to the site of hydrolytic breakdown of nucleic acids. *Z. Zellforsch. Mikrosk. Anat.* **80:** 353–369.